# THE OCCURRENCE OF β-ALANINE AND β-AMINOISOBUTYRIC ACID IN FLATWORMS

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β-Alanine and β-aminoisobutyric acid were first shown to be products of uracil and thymine metabolism, respectively, in the rat by Fink and co-workers (1956). Recently, the pathway for the formation of these β-amino acids from pyrimidines has been implicated as part of a celluar homeostatic mechanism which is involved in the regulation of ribonucleic and deoxyribonucleic acid synthesis (Canellakis *et al.*, 1959). Since β-alanine and β-aminoisobutyric acid were found to be prominent free amino acids in three species of cestodes (Campbell, 1960a) and have subsequently been shown to arise metabolically from pyrimidines *via* the pathway elucidated in the rat in a fourth species (Campbell, 1960b), the study of the distribution of these two compounds in flatworms was extended to include other symbiotic and free-living species.

β-Alanine, a product of uracil metabolism, was first reported as a decarboxylation product of aspartic acid in bacteria by Virtanen and Laine (1937). It has since been reported to occur in the free state in certain mammalian tissues (Tallen *et al.*, 1954; Roberts *et al.*, 1950), insects (Clark and Ball, 1951; PoChedley, 1956), various plant tissues (Hulme and Arthington, 1950; Synge, 1951), and recently in several species of mollusks (Simpson *et al.*, 1959). β-Aminoisobutyric acid, a product of thymine metabolism, was first isolated from human urine by Crumpler *et al.* (1951). It has since been reported in tissues of the cat (Tallen *et al.*, 1954), the iris plant (Asen *et al.*, 1959), and the mollusk, *Mytilus edulis* (Awapara and Allen, 1959).

## Materials and Methods

The preparation and extraction of the cestodes was carried out as previously described (Campbell, 1960a). The free-living species and the symbiotic species which have a digestive tract were either kept in the laboratory for a sufficient length of time for the tract to empty normally or this was hastened by applying pressure to the organisms with a glass rod. They were then homogenized and extracted by the method used for the cestodes. Total nitrogen in the extracts was determined by a micro-Kjeldahl method (Lang, 1958). The final solution of the free amino acids was made up to contain one mg. nitrogen per ml. Fifty to 300 microliters of this extract were then spotted on Whatman No. 52 paper for chromatography. Two-dimensional chromatography was carried out by the method of Levy and Chung (1953) with the substitution of a sec-butyl alcohol: formic acid: water sol-

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vent system (Hausmann, 1952) in the first dimension. The papers were developed two times in the first dimension. The lowest limit of detection for these two compounds is approximately 0.05 micromoles; consequently, a concentration of β-alanine or β-aminoisobutyric acid in the extracts of less than 0.05 micromoles per 300 microliters extract would not be detected. After the initial chromatography indicated that β-alanine and/or β-aminoisobutyric acid were present, the following methods were used to obtain chromatographic evidence for the identity of these two compounds. In most cases the free amino acid extracts of the flatworms which contained β-alanine and/or β-aminoisobutyric acid were further separated into a basic, neutral, and acidic fraction with Amberlite CG-50 $H^+$ and Dowex 50 $H^+$ (Simpson *et al.*, 1959). The neutral fraction contained the β-amino acids. Known β-alanine and β-aminoisobutyric acid added to this fraction or to the extracts gave no additional spots upon two-dimensional chromatography and there was an intensification of the unknown spots in the extracts which were thought to be β-alanine and β-aminoisobutyric acid. The β-amino acids were then separated

Table I

*$R_f$ values of known β-alanine and β-aminoisobutyric acid and compounds isolated from flatworms*

| | $R_f$ | |
|---|---|---|
| Solvent system | β-Alanine | β-Aminoisobutyric acid |
| (1) sec-butyl alcohol:formic acid:water (75:15:10, by vol.; developed 2x) | 0.52 | 0.67 |
| (2) m-cresol:phenol:borate buffer, pH 8.3 (30:15:7.5, w/w/v) | 0.41 | 0.53 |
| (3) phenol:water (72:28, v/v) | 0.66 | 0.76 |
| (4) lutidine:water (62:38, v/v) | 0.30 | 0.35 |
| (5) methyl alcohol:pyridine:water (80:4:20, by vol.) | 0.49 | 0.58 |

Whatman No. 52 paper (solvents 1 and 2) and Whatman No. 3 MM paper (solvents 3, 4 and 5)

from the remainder of the amino acids by streaking the neutral fraction or the extracts on Whatman No. 3 MM paper, chromatographing the papers one-dimensionally in sec-butyl alcohol: formic acid: water two times, and eluting the appropriate portion of the papers to recover the β-amino acids. These eluates, which contained a single β-amino acid, were then concentrated and run in several solvent systems with and without added known β-alanine and β-aminoisobutyric acid. These known compounds were also run as reference compounds by themselves. Identical $R_f$ values were obtained with five solvent systems for both the known compounds and the compounds isolated from the worms. These data are given in Table I. Additional evidence for the nature of the compounds was obtained by pre-treatment of the paper with alkaline copper carbonate. This treatment did not hinder the movement of these compounds during chromatography. Crumpler and Dent (1949) have shown the α-amino acids are rendered immobile as far as chromatography is concerned by the formation of a copper complex, whereas the $R_f$ values of β-amino acids remain relatively the same in the presence of copper.

## Results and Discussion

The results of this survey are presented in Table II. β-Alanine and β-aminoisobutyric acid have been identified as free amino acids in all the species of cestodes thus far examined. In *Hymenolepis diminuta,* the concentrations of β-alanine and β-aminoisobutyric acid are approximately equal, amounting to $0.60 \pm 0.04$ and $0.65 \pm 0.08$ micromoles, respectively, per gram of tissue (determined by the method of Fowden, 1951). On the basis of the relative intensities of the ninhydrin reaction, β-alanine occurs in higher concentration than β-aminoisobutyric acid in the other four species of cestodes. In *Macrapis cristata,* β-aminoisobutyric acid is in higher concentration than β-alanine. In *Bdelloura candida* and *Entobdella bumpusi,* the two are approximately equal. In this survey only two strictly free-living species were examined, consequently no generalizations can be made concerning the occurrence of these two compounds in symbionts *vs.* free-living species. However, these data indicate that β-alanine and β-aminoisobutyric acid are quite common free amino acids in symbionts which are in contact with host tissue. A notable exception in this case is the liver fluke, *Fasciola hepatica,* which does not have either β-amino acid. It has been shown (Campbell, 1960b) that *H. diminuta* degrades uracil and thymine to β-alanine and β-aminoisobutyric acid, respectively, *via* the intermediate dihydropyrimidines and carbamoyl-β-amino acids which were first shown to be intermediates in rat liver (Fink *et al.,* 1956). Since this path-

Table II

*The distribution of β-alanine and β-aminoisobutyric acid in flatworms*

| Species | Habitat | β-Alanine | β-Aminoisobutyric acid |
|---|---|---|---|
| Turbellaria: | | | |
| *Bipalium kewense* | Free-living (land) | –* | – |
| *Dugesia tigrina* | Free-living (fresh-water) | – | – |
| *Stylochus zebra*** | Symbiotic with hermit crab (*Pagurus*) | – | – |
| *Bdelloura candida* | Symbiotic upon horseshoe crab (*Limulus*) | +* | + |
| Trematoda: | | | |
| *Fasciola hepatica* | Endoparasitic in liver of cattle | – | – |
| *Fascioloides magna*** | Endoparasitic in liver of cattle | + | – |
| *Macraspis cristata* | Endoparasitic in liver of sting ray (*Dasyatis centrura*) | + | ++ |
| *Entobdella bumpusi* | Symbiotic upon sting ray | + | + |
| Cestoda: | | | |
| *Hymenolepis diminuta* | Endoparasitic in gut of rat | + | + |
| *Calliobothrium verticillatum* | Endoparasitic in gut of dogfish shark (*Mustelus canis*) | ++ | + |
| *Lacistorhynchus tenuis* | Endoparasitic in gut of dogfish shark | ++ | + |
| *Phyllobothrium foliatum* | Endoparasitic in gut of sting ray | ++ | + |
| *Disculiceps pileatum* | Endoparasitic in gut of dusky shark (*Carcharhinus obscurus*) | ++ | + |

* + denotes that the compound was present; – denotes that it could not be detected; ++ denotes that this compound occurs in higher concentration.

** Only one specimen available for analysis.

way may also be present in the other species of flatworms which have these β-amino acids, the presence or absence of these compounds may reflect the availability of pyrimidines or pyrimidine-containing compounds in the environments of the worms. Another possibility is that the species which do not contain β-amino acids as free compounds are incapable of degrading uracil and thymine. This latter possibility is not known at the present.

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## Summary

A survey of thirteen species of free-living and symbiotic flatworms for β-amino acids has been carried out. β-alanine and β-aminoisobutyric acid were found to occur most commonly as free amino acids in the symbiotic species in contact with the tissues of their host. These compounds were not detected as free amino acids in the two free-living species which were examined.

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